ACCELERATED CLOTTING TIME AS A DETERMINANT OF FOETAL MATURITY AND ITS VALUE IN DIAGNOSING POST DATED PREGNANCY.

THESIS

FOR

MASTER OF SURGERY

(OBSTETRICS & GYNAECOLOGY)

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JHANSI (U. P.)

1993 SHUBHA DIXIT

C E R T I F I C A T E

This is to certify that the work entitled "ACCELERATED CLOTTING TIME AS A DETERMINANT OF GESTATIONAL AGE AND ITS VALUE IN POST DATED PREGNANCY", which is being submitted as a thesis for M.S. (Gynaecology & Obstetrics) by Dr. Shubha Dixit has been carried out under my direct supervision and guidance in the department of Gynaecology & Obstetrics. The technique embodied in the thesis were undertaken by the candidate herself and the observations recorded have been periodically checked and verified by me.

She has put in the necessary stay in the department as per University regulations.

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CONTENTS

INTRODUCTION

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Correct timing of delivery to avoid complications of prematurity or of suspected post dated pregnancy and proper timing of termination of high risk pregnancies has remained a major obstetrical problem.

Clinical criteria like LMP, Date of quikening, fundal height at per abdominal examination has been inadequate. Survival after delivery depends on functional development rather than chronological age.

There has been development of many methods to assess the gestational age of which main methods are -

1) Radiological methods
2) Ultrasonography
3) Analysis of amniotic fluid.

Amniotic fluid has been vastly studied for assessment of foetal maturity. Such as L/S ratio indicating lung maturity, creatinine showing kidney maturity, cytology suggesting skin maturity.

In recent years a number of components of the amniotic fluid have been noted to change progressively during pregnancy and accordingly have been intensely investigated as indicators of fetal maturity. Its constituents vary with period of gestation and, therefore, most correctly reflect physical and functional status of the fetus.

The various methods evolved for assessment of foetal maturity by amniotic fluid examination are -

1. Volume estimation
2. Cytological examination
3. Creatinine levels
4. Urea levels
5. Bilirubin levels
6. Osmolarity
7. Phospholipid levels
8. Biochemical analysis
9. Shake test or Bubble stability test
10. Accelerated clotting time (ACT)

Since 1926, when Meyer first described the symptoms of material respiratory distress and cardiovascular shock associated with the infusion of amniotic fluid during labour. The procoagulant properties of amniotic fluid were studied.

Thromboplastic activity of amniotic fluid has been held responsible by number of workers in last decade for intra-vascular microembolization specially in lungs, in cases of amniotic fluid embolism.

Hastwell in 1974 evaluated a bedside test based on thromboplastic activity of amniotic fluid. He suggested that as the period of gestation increases thromboplastic activity of amniotic fluid increases and clotting time decreases. Acceleration of clotting process has been termed by him as (ACT). Present study is based on same principle to find out foetal maturity and its correlation with ACT by using amniotic fluid.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

1) Origin of Amniotic fluid
2) Study of various properties in relation to gestational age
3) L.S. ratio, Bubble stability test, cytology
4) Procoagulant properties of Amniotic fluid
5) Accelerated clotting time.

The origin of Amniotic fluid :

The physiological process involved in production of amniotic fluid are still not clear and investigations are complicated by the multiple sites of formation. The fluid could arise as a secretion or an ultrafilterate either from the mother across the membranes, from the placenta and cord or from the fetus through the skin, gastro-intestinal tract, tracheobronchial tree or kidneys.

In early part of this century, it was commonly believed by physiologists, mainly from the experiments of Holtermann (1924), that fetal kidneys were not active in utero. There is now overwhelming clinical and experimental evidence

for intrauterine fetal urine production starting as early as the end of first trimester (Abramovich 1968).

The association of polyhydramnios with haemangiomatous tumour of chorion could be interpreted as evidence of increased liquor production at the site.

Oligohydramnios is associated with the condition of amnio nodosum where the amniotic epithelium is histologically deranged and Landing (1950) felt that this represented a loss of secretory function.

The increasing biochemical resemblance of liquor to fetal urine, particularly after keratinisation of the fetal skin, is seen by increasing concentration of urea, uric acid and creatinine, decreasing sodium and glucose and also by reduction in osmolality (Pauleen 1955).

The presence of amniotic fluid in the early embryo when the tissue of the fetus is poorly differentiated and non-functional and in the cases

of some blighted ova where the fetus is rudimentary or absent, suggest that initially the amniotic fluid enters via the membrane (Jeffcoate & Scott 1959).

Compbell et al (1973) visualised the fetal bladder using ultrasound screening and demonstrated 'in utero' bladder emptying. They were able to calculate hourly fetal urine production.

The role of fetal respiratory tract in the formation of liquor is much less fully substantiated. The appearance of fetal surfactant in the liquor has become an important clinical test in assessing fetal maturity (Cluck et al 1971, Bhagwani et al 1972) and some proteins particularly albumin may enter the liquor through the respiratory tract (Scarpelni 1975). Studies of biochemical and immunological constituents of the amniotic fluid might also suggest that liquor is derived from maternal plasma since much of the soluble proteins is of maternal origin (Sutcliffe 1975).

However, larger molecules, particularly the globulin have been shown to enter the fetus by direct transport across the chorionic villi (Gitlin and Gitin 1976) and are secreted into the liquor in fetal urine.

Several other possible sources of fetal secretions which might contribute to the amniotic fluid include the gastro-intestinal tract, the salivary glands and the buccal mucosa.

Several workers have studied the various components of amniotic fluid and changes in these substances in relation to gestational age. Thus various parameters emerged as criteria for determining gestational age of which important ones are determination of lecithin/sphingomyelin ratio creatinine, urea, amylase in amniotic fluid cytology and bubble stability test.

Fetal Pulmonary maturation and surfactant production and bubble stability test :

The initial development of the bronchial tree, from the appearance of the endodermal lung bud (at 24 days of foetal life) to the formation of the terminal conducting bronchioles (at 16 weeks), is followed by an intermediate phase during which respiratory bronchioles are formed and then by a final stage of Alveolar development which beings at

24 weeks. During the final phase, the alveolar duct and sacs form, the alveolar lining membrane, differentiates into type 1 and type 2 pneumocytes and surfactant is produced by type 2 cells. It is the appearance of surfactant at the start of this final phase that marks the begining of functional pulmonary development and makes possible the maintanence of alveolar expansion and ventilation, so that, in the event of birth, survival is now possible.

Preliminary studies by Holmy & Hack (1962) had demonstrated differences between the lipids in maternal and cord blood and of human amniotic fluid. Although the phosphatide pattern of amniotic fluid showed a strong resemblance to plasma, there were several marked differences, and they concluded that the lipids of amniotic fluid had a mixed origin.

Only small amounts of alveolar surfactant, the active component of which are the phospholipids lecithin and sphingomyelin, are present in lungs of very premature infants (Adams et al 1965), but there

is progressive increase in the amount towards term (Cluck et al 1967). Between the first trimester and term there was approximately a two fold increase in the phospholipid concentration, where as initially lacithin accounted for between 28 and 51% of the total phospholipid, at term this proportion had increased to between 50 - 79%. The contribution of sphingomyelin, however, fall from 29-51% to 25-46%.

In 1972 Clements et al reported a simple beside test using the minimum of apparatus and materials with high predictive value for the respiratory distress syndrome when variously referred to as the shake test. Foam stability test, bubble stability test (B.S.T.) or rapid surfactant test.

Sharma et al (1981) compared the three parameters L/S ratio, shake test and T.P.P. with each other for assessment of fetal maturity. Their study suggested, that T.P.P. values are more reliable prior to 32 weeks when lecithin is low. While after that L/S ratio and shake test are more informative. Shake test can be used as a screening procedure and more elaborate L/S ratio can be estimated when very

small bubbles are obtained or bubble clumping occurs or in intermediate test to differentiate the possibly immature fetus from the likely mature fetus and in those cases showing a deviation from normal.

Cytology of amniotic fluid :

It has long been known that fetal epidermal cells are absent from the amniotic fluid until late in pregnancy, but only in the last 15 years has this found a clinical application in determination of gestational age or fetal maturity.

Nile blue sulfate staining was first applied in the diagnosis of ruptured membranes by Kittrich in 1963. It was described by him as follows. To the unfixed, wet vaginal smear a few drops of aqueous nile-blue sulfate solution are added. After 3 - 5 minutes the slide is covered with a coverslip and examined. The oxazene present in the staining solution.stains neutral fats red. As epidermal cells desquamating from fetal skin are covered to a large extent with vernix caseosa.

They will appear as orange - yellow elements between the blue vaginal cells, free fat droplets may be encountered apart from the foetal cells.

In amniotic fluid stained with nile blue. Sulfate three main types of cells were observed a large pale blue and often polygonal cells with the morphological characteristics of a superficial squamous cell, clearly derived from the fetal sequamous epithelium, a small round cell with deeply staining blue cytoplasm and with a larger nucleus derived from the lining epithelium.

A third type of cell, the 'fat cell', which stained orange to brown, either ovoid or polygonal in shape and present in varying numbers depending on the duration of pregnancy.

The ratio of the amnion cells to superficial squamous cells decreased after 32nd week of pregnancy. Towards the end of pregnancy, as fat cells rose rapidly a tendency to form clusters became more apparent and it was only possible to make a rough estimate of cells contributing the clusters.

Fluids obtained per vaginum have to be examined immediately because of the rapid deterioration of cellular components due to bacterial contaminants.

Assessment of fetal maturity by nile blue sulfate staining of liquor amnii was done by Rajee Iyer (1981). An orange stained lipid laden cell count of over 10% always suggested a maturity of over 38 weeks gestation. However, a lower percentage count (1-9%) when associated with plenty of droplets (6-8 per low power field) was also compatible with a gestation of over 38 weeks. Majority of samples over 38 weeks of pregnancy gave a count in the range of 10-30% with a few being in the 31 - 80% range. In post-mature pregnancies, counts varying between 30 - 50% were recorded in the present study.

De Castro et al (1975) evaluated the most reliable means of determining fetal maturity. They analysed six amniotic fluid components from 99 women who were from 22 to 40 weeks pregnancy. The six parameters investigated were, amylase activity, the L/S ratio, percentage of fat cells and the

concentration of protein creatinine and bilirubin. In general, single components and combinations of two or three of the tests did not significantly enhance the predictive accuracy or reduce the error from that obtained with L/S ratio used alone.

The Procoagulant activity in the Amniotic fluid :

The procoagulant activity of amniotic fluid is clearly demonstrated in various studies. Studies show that addition of amniotic fluid bypasses the coagulation factors XI, IX and VIII in the intrinsic system and that its action is at a further lower level of the coagulation cascade.

As regards the extrinsic system of blood coagulation, the P.T. (Prothrombin Time) as well as R.V.V. time (Ressel Viper Venom) was reduced to a very small extent after the addition of amniotic fluid (Rendelstein et al 1951; and Phillips and Davidson 1972). This indicates that the amniotic fluid acts in a manner very akin to tissue thromboplastin.

The addition of amniotic fluid corrects only partially the recalcification time as well as the prothrombin time of factor VII + X deficient substrate plasma. The mean prothrombin time of 90 seconds was reduced to 53.30 seconds on the other hand the R.V.V.- Cephalin time of similar substrate plasma samples was reduced to a very small degree i.e. from a mean of 40 second to 36.1 seconds. Since the action of russel viper venom does not require factor VII in contradiction to that of tissue thromboplastin. The difference on the activity by these two tests on VII/X deficient substrate plasma would indicate that amniotic fluid bypasses most of the activity of factor VII and that its main action is on the activation of factor X.

Phillips and Davidson (1972) also had similar results with prothrombin time and R.V.V. - Cephlin time using substrate plasma deficient in factor VII and X. They also used substrate plasma deficient in factor VII only and demonstrated marked lowering of recalcification time as well as R.V.V. time. On the

other hand, with factor X deficient plasma the addition of amniotic fluid brought about rather an increase in R.V.V. time.

Since factor V is below factor X in the coagulation scheme, the substrate plasma deficient in the former would not be affected by the addition of amniotic fluid if its major action was the activation of factor X.

Experiments with whole amniotic fluid (uncentrifused) and with supernatant of centrifused amniotic fluids showed little difference in procoagulant activity in these two types of samples. It can thus be inferred that the procoagulant activity is mostly present in the soluble form. Phillips and Davidson (1972) fractionated the lipid contents of the amniotic fluid. They demonstrated the presence of phosphatidyl ethanoplamine and these lipids could very well be expected to shortened the recalcification time.

It is probable that a part of thrombokinase like activity comes from the decomposed material of

epithelial cells of embryonic skin and amnion. A part of similar activity may also come from fetal urine in the amniotic fluid (Rendelstein et al 1951).

The clear supernatant amniotic fluid after centrifugation does not possess any platelet aggregating activity neither any inhibiting material to A.D.P. was demonstrable. However, since particulate material is present in abundance in amniotic fluid, that may be of crucial significance in initiating platelet aggregation ultimately leading to disseminated intravascular coagulation (McKay 1959).

The procoagulant activity of amniotic fluid is probably physiologically important in maintaining the hemostasis at the time of normal placental separation female patients with factor XI deficiency seldome have postpartum hemorrhage. On the contrary, those with factor V deficiency have frequent problem of postpartum bleeding (Phillips and Little 1962). This indicates the role of thrombokinase like activity of amniotic fluid and normal local hemostasis during parturation.

Hence the amniotic fluid is shown to possess potent procoagulant activity which increases with the advancement of pregnancy.

Accelerated Clotting time :

Thromboplastins are substances which accelerate the clotting process by promoting the conversion of prothrombin to thrombin. These substances are present in all cells. Particularly in tissues with a high lipid content, such as brain, placenta, lungs and testis. Thromboplastins are lipoproteins composed of a phospholipid and protein moiety. The phospholipid moiety is relatively heat stable, but the protein moiety is heat labile.

Injured and degenerating cells release thromboplastins as their lipoprotein membranous endoplasmic reticulum and microsomal structures become distrupted. Aging fetal cells desquamate in increasing numbers as the fetus matures and release thromboplastins. Any condition severe enough to injure placental and fetal cells would be expected to release thromboplastins.

The test for accelerated clotting time probably measured the combined thromboplastic activity of the dequamated cells and phospholipids in the amniotic fluid.

ACT is a simple, rapid and useful test in providing additional information in relation to fetal maturity and the optimum time for induction of labour.

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MATERIAL & METHODS

MATERIAL & METHOD

This study was carried out in patients attending Maharani Laxmi Bai Medical College Hospital, Jhansi for confinement (both normal and abnormal pregnancy) in a study period of 12 months.

SELECTION OF CASES :

The study group comprised of cases attending Out Patient Department of Obstetrics and those hospitalised for obstetrical conditions. The cases included patients with normal pregnancy. Patients with pregnancy with complications like pre-eclamptic toxemia, Rh incompatibility hydramnios, hydrocephalous, twins who came for antenatal check up and for delivery in the hospital. These cases were further evaluated under following headings.

A. Clinical Presentations :

1. History : Detailed history of present pregnancy was taken trimesterwise and precise date of last menstrual period was documented to know the exact period of gestation obstetrical history and detailed family history of twins, hydramnios and congenital malformations was also taken.

2. General Examination : Thorough general examination was done with special attention and regards to pulse, blood pressure, pallor, cyanosis, jaundice and edema.

3. Brief Systemic Examination of cardiovascular and respiratory system was done.

4. Abdominal Examination was done to determine the approximate duration of gestation, position and condition of the fetus. Any evidence of hydramnios and twins was looked for. Foetal Heart Sounds were heard to detect any evidence of foetal distress.

5. Per vaginum Examination : At term per vaginum examination was performed to know the dilatation of cervix. Progress of labour to detect the presence of bag of membranes, leaking and to detect presence of muconium.

All cases of normal and abnormal pregnancy were put in 4 groups according to gestational period which was documented by knowing the LMP and per abdominal and per vaginal examination as and when needed.

(i) Group A : Comprising of gestation period ranging from 28 - 32 weeks.

(ii) Group B : Comprising of gestation period ranging from 32 - 36 weeks.

(iii) Group C : Comprising of gestation period ranging from 36 - 40 weeks.

(iv) Group D : Comprising of gestation period ranging from 40 - 42 weeks.

(v) Group E : Comprising of gestation period ranging above 42 weeks.

B. Pathological Examination :

In each case following investigations were performed :

1. Complete haemotological examination including total and differential leucocyte counts, haemoglobin percentage and erythrocytic sedimentation rate.

2. Blood ABO and Rh grouping of mother and if indicated of father. Rh antibody titre was done serially, when indicated.

3. Urine analysis for the presence of albumin, sugar and microscopic examination.

4. Fasting and post-prandial blood examination, when indicated.

METHODS OF COLLECTION OF AMNIOTIC FLUID :

a) Per vaginally

b) Trans abdominal amniocentesis

c) During caesarean section

a) Per vaginum amniocentesis :

This was done by inserting a needle with syringe under full aseptic conditions through the bulging membranes in cases who came in labour after two finger dilation of cervix. Samples were also collected at the time of spontaneous rupture of membrane or at the time of artificial rupture of membrane. Precautions were taken not to contaminate the samples with vaginal discharge, urine or blood.

b) Trans abdominal Amniocentesis :

Preparation of the patient :

* A written consent of patient was taken. She was asked to evacuate her bladder. Cases who were unable to evacuate the bladder were catheterised.

* In very apprehensive patients injection diazopam (10 mg) was given, intramuscular 30 minutes before doing amniocentesis.
* Patients were made to lie down in dorsal position with legs slightly flexed at the hip joint and head slightly raised.
* In un-cooperative patients 1% solution of local anaesthetic drug (xylocain 1%) was injected locally at the selected point.
* Abdomen was painted with antiseptic solution and draped with autoclaved sheets.

Technique of Amniocentesis :

Abdominal examination was carried out to know the presentation and position of foetus. Foetal heart sounds were counted and their regularity was noted. A point was selected midway between the anterior superior iliac spine and umbilicus, between the gap of upper and lower limbs of the foetus or any point in the mid line suprapubically. Under all aseptic

precautions a 21 gauge, 4 inch spinal needle was directly introduced into the uterus. Stellate was removed and point was sealed with tincture benzoin.

Amniotic fluid specimen were placed in clean test tube and labelled. Patients were asked to remain in bed for 2 hours and were watched for pulse and blood pressure. Foetal heart sounds were auscultated for rate and regularity.

c) During Caesarean Section :

During lower segment caesarean section, 5 ml of amniotic fluid was collected with the help of autoclaved syringe and needle after incising the visceral peritoneum just before giving incision in the uterus.

Determination of Accelerated clotting time and control clotting time :

Method used was given by Hastwell (1974)

A sample of blood was rapidly drawn from patients cubital vein and 1.5 ml was added immediately to 1 ml of fresh amniotic fluid at body temp. 37^{o}C.

The temperature was maintained by immersing the collection tube in a waterbath.

The clotting time was noted, the tube being tipped on to its side every 5 seconds, this time was termed accelerated clotting time.

A control clotting time was obtained by placing 2.5 ml of the same sample of blood into a similar tube and noting the whole blood clotting time in the same manner. Meconium and Blood stained liquor sample was discarded.

If the control was between 3 and 6 min, the conditions of the test were considered satisfactory.

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OBSERVATIONS

OBSERVATIONS

The present study consists of cases admitted in M.L.B. Medical College Hospital, Jhansi in the Department of Obstetrics & Gynaecology during the study period of August, 1991 to April, 1992.

These cases were divided into 5 groups according to period of gestation.

Group - I : Comprising of pregnancies of 28-32 weeks gestation.

Group - II: Comprising of pregnancies of 32-36 weeks gestation.

Group -III : Comprising of pregnancies of 36-40 weeks gestation.

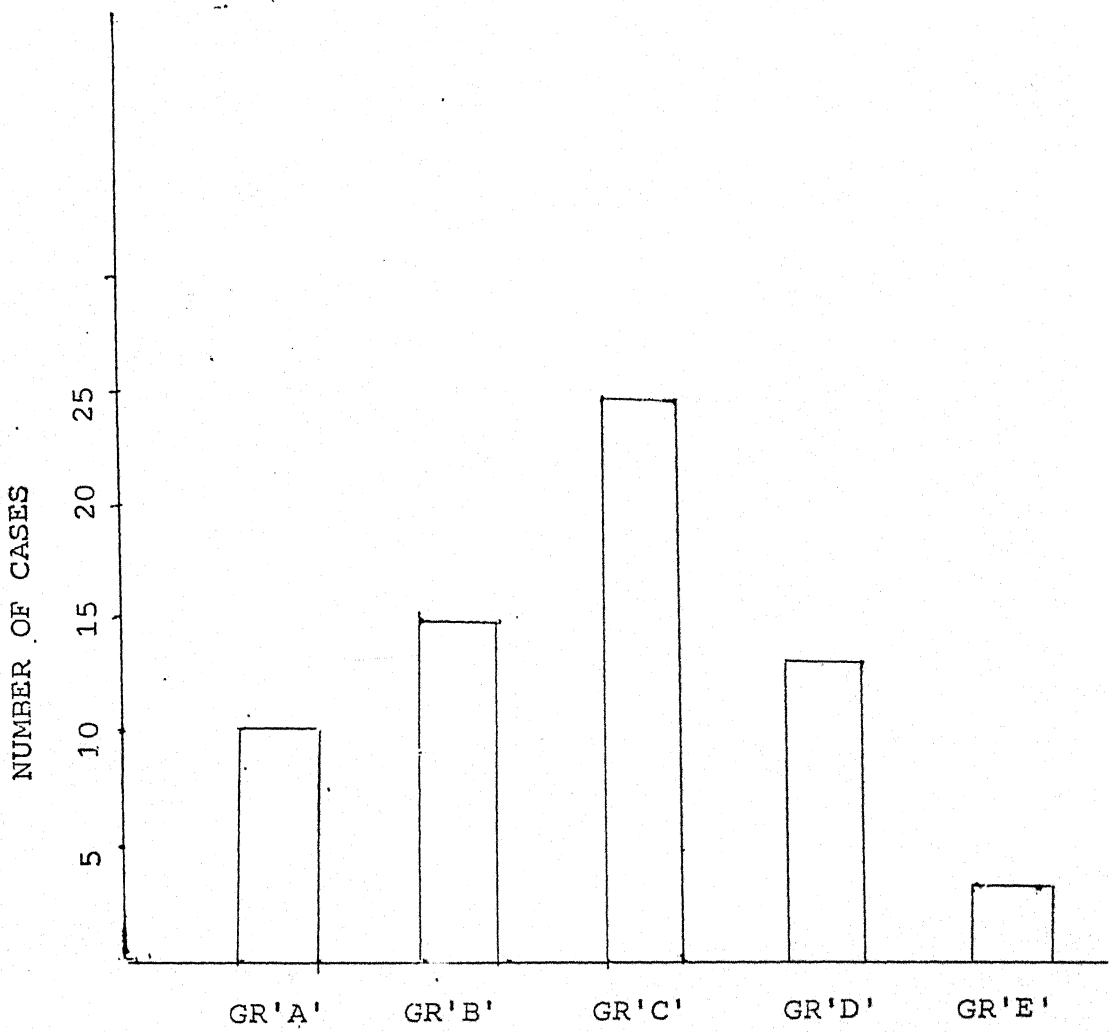
Group - IV : Comprising of pregnancies of 40-42 weeks gestation.

Group - V : Comprising of pregnancies of 42 weeks or above.

The distribution of number of cases in each Group is shown in table - I.

TABLE - I

DISTRIBUTION OF CASES



These patients were usually admitted in late pregnancy or labour. In all these cases control clotting time and accelerated clotting time was determined using fresh amniotic fluid and blood drawn from antecubital vein of the mother. The gestational age was seen by using physical criteria to determine age of newborn.

This study suggests that there is a Linear fall of ACT as the duration of gestation increases.

In group - I : the period of gestation varied between 28-32 weeks , the range of ACT was between 130 - 200 Sec.

Average ACT was 169.6 Sec with SD 22.4.

i.e. ACT varied between 169.6 $\pm$ 22.4 Sec.

In group - II : where the period of gestation varied between 32-36 weeks the range of ACT was between 96 - 145 Sec.

Average was 117.67 Sec with SD 15.03.

i.e. ACT varied between 117.67 $\pm$ 15.03 Sec.

In group -III : where the period of gestation varied between 36-40 weeks the range of ACT was between 50 - 112 Sec.

Mean was 80.44 Sec with SD 17.5

i.e. ACT varied between 80.4 $\pm$ 17.5 Sec.

Comparing these three pair of Data it is seen clearly as the gestational age is increasing, there is clear fall of ACT.

By simple analysis ACT of 117 or less can be taken as an index of foetal maturity. ($p < .001$) i.e. highly significant.

In group - IV : where the period of gestation was 40 to 42 weeks, the range of ACT was 46 to 68 Sec. and average was 51.9 Sec with SD 16.1

i.e. ACT varied between 51.9 $\pm$ 16.1 sec.

In group - V : where the period of gestation was 40 weeks or more, the ACT ranged between 44-54 Seconds and Mean was 48 Sec with SD 5.29

i.e. ACT varied between 48 $\pm$ 5.3 sec.

By analysing this data statistically though after 40 weeks with continuing pregnancy, there is clear fall in ACT and statistically it is significant ($P < .05$).

It can be inferred that there is Linear fall in ACT as the gestation increases and value of 51.9 sec

TABLE - II

Group	Period of gestation (in weeks)	No.of cases	Range of ACT (Secs)	Average ACT (Secs)	SD	Statistical comparison		
						Comparison between Groups	t	P
I	28 - 32	14	130-200	169.6	22.4	I & II	6.99	$<.001$
II	32 - 36	15	95-145	117.67	15.03	I & III	12.57	$<.001$
III	36 - 40	25	50-112	80.44	17.5	I & IV	14.68	$<.001$
IV	40 - 42	13	46- 68	51.9	16.09	I & V	9.434	$<.001$
V	$\nearrow$ 42	03	36- 48	46.0	05.29	II & III	6.87	$<.001$
						II & IV	11.2	$<.001$
						II & V	8.13	$<.001$
						III & IV	4.89	$<.001$
						III & V	3.29	$<.01$
						IV & V	6.42	$<.05$

is significant to show fetus is above 40 week but after that though the ACT still falls it is not very significant.

Hence the value of ACT is to detect a post dated pregnancy but it will not differentiate a postmature fetus, from post dated one.

Hence by statistical methods the different groups of compared and it can be inferred that ACT less than 98 seconds indicates mature fetus as shown in table II.

It is a well known fact that birth weight is related to gestational age. As the gestational age increases the weight of the baby also increases in a normal pregnancy. Thus there is a Linear positive correlation of birth weight with gestational age.

In the present study also we have studied physical criteria like ear cartilage, scalp hair, breast nodule, genitalia and sole creases and also the birth weight to ascertain the gestational age.

The observations are compiled in table III.

All of the cases are divided according to birth weight of the baby.

Group - I : Consists of foetus of less than 1000 gm at birth.

Group - II : Consists of babies ranging 1000gm to 2500gm at birth.

Group - III : Consists of babies ranging 2501 gm to 3500 gm at birth.

Group - IV : In this group babies who were more than 3501 gm were included.

It was seen that in babies of less than 1 kg at birth the ACT ranged between 126 - 180 seconds. The mean ACT being 153.43 second with standard deviation of 19.5.

In group - II : in babies ranging between 1 kg to 2.5 kg at birth, the accelerated clotting time (ACT) was between 106-60 seconds. The mean ACT was 68.63 secs with standard deviation 19.94 i.e. ACT was 68 $\pm$ 19.94 secs.

In group - III: the ACT was between 46-60 sec. Average being 56.8 seconds with standard deviation 17.18.

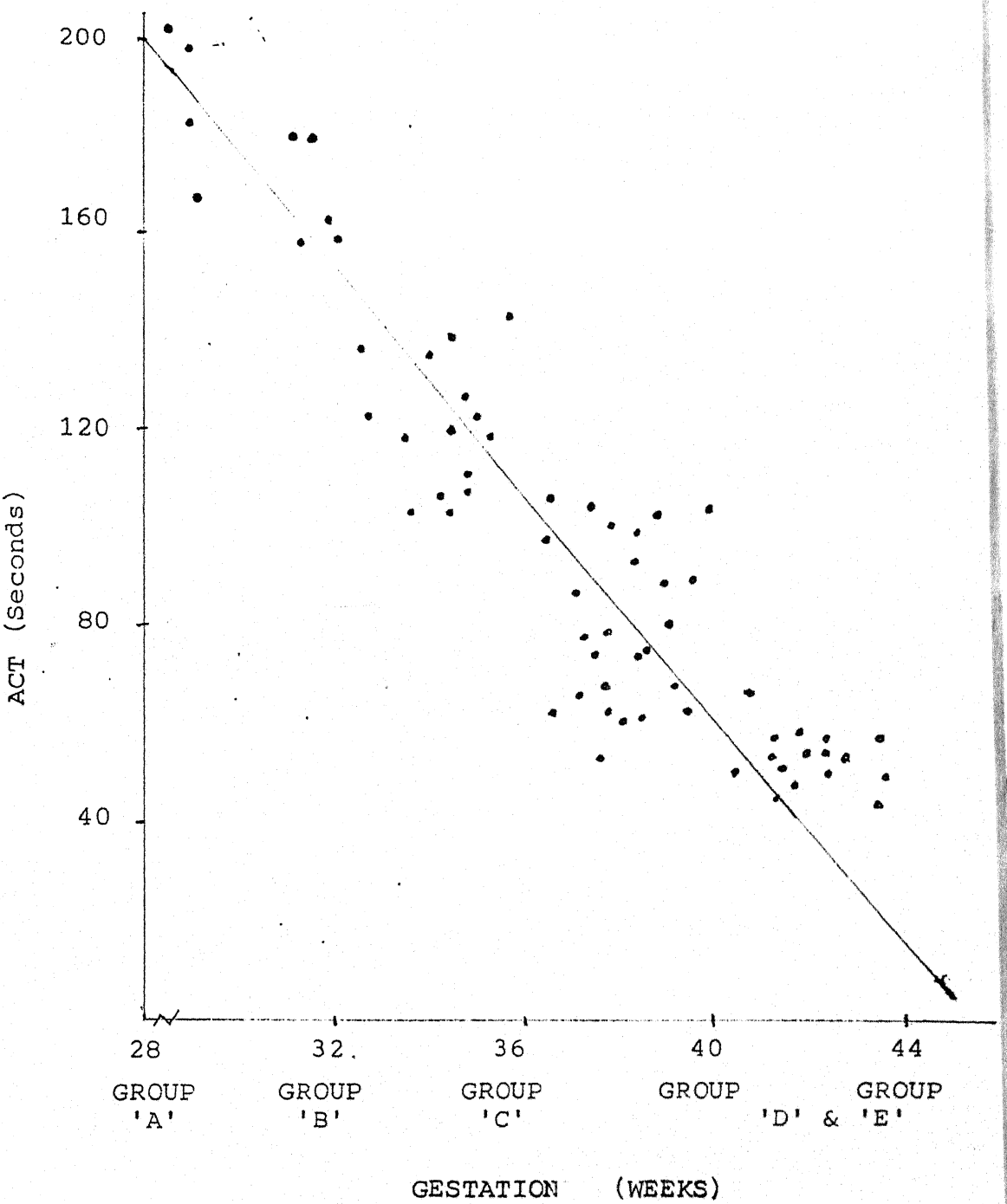
Most mature foetus with good APGAR scores were found to be in group II & III only indicating the ACT of less than 90 sec can be taken as an index of foetal maturity.

In group-IV : babies born with birth weight of 3.51 kg or more were included which show ACT ranging 40 - 56 sec with average being 49 second and standard deviation 2.75.

On application of statistical methods and comparison of various groups the difference in ACT of different groups were found to be highly significant at $p < .001$ as shown in the table - III.

TABLE - III

Group	Birth weight in Gms.	No.of cases	Mean ACT in sec.	S.D.	Statistical comparison		
					Groups compared	t	p
I	⩽1000	16	153.43	19.5	I & II	13.53	⩽.001
					I & III	19.13	⩽.001
II	1000-2500	19	68.6	19.94	I & IV	12.95	⩽.001
					II & III	27.15	⩽.001
III	2501-3500	22	56.8	17.18	II & IV	2.38	⩽ .05
IV	3501-4000	13	49.0	2.75	III & IV	1.106	⩾.05



SCATTER DIAGRAM SHOWING FALL OF ACT (ACCELERATED CLOTTING TIME) AS GESTATIONAL AGE INCREASES.

DISCUSSION

DISCUSSION

This study was done in 70 patients to determine a relation between accelerated clotting time and gestational age in the department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi. The results of the present study suggest a significant relationship between accelerated clotting time and increasing fetal maturity.

Accelerated clotting time is a measure of procoagulant activity of amniotic fluid. It is found by adding fresh amniotic fluid to freshly drawn maternal blood. A control is also set up with same blood specimen. It was found that addition of amniotic fluid to blood decreases the clotting time.

In the present study, there was a significant decrease in ACT (Accelerated clotting time) as is shown visually in the scatter diagram. This decrease in ACT suggests an increase in thromboplastic activity in amniotic fluid and increasing gestational age. Similar findings have also been reported by Hastwell (1974 & 1978).

Karna et al 1984 suggested ACT (Accelerated Clotting Time) of 115 seconds or less can be taken as index of foetal maturity. Of the 50 cases studied there was only 1 case of false +ve (positive) where ACT was 108 and gestation was 36 weeks i.e. 2%.

In the present series ACT of 98 seconds or less can be taken as index of foetal maturity i.e. gestational age of more than 36 weeks.

Only one case of false positive was found where ACT was 96 and gestational age was 34 - 35 weeks.

Hastwell in 1974 and 1978 suggested ACT of less than 110 seconds usually indicated mature foetus of more than 36 weeks. He found good correlation of ACT L/S ratio and gestation.

Hastwell studied 11 patients serially in the last trimester. He showed there was a decrease in ACT and, hence an increase in thromboplastic activity, with increase in maturity. In these series he could study the effect of maturity in the individual patient, he could study 2-3 samples from 30th to 42nd weeks. In this series also the ACT in patients after 38 weeks was below 120 seconds.

Based on this study the (Linear) formula for prediction, based on Linear regression was found to be -

$$Y = 45 - .07 X$$

where Y is age in weeks and X = ACT

The Linear relationship has the advantage of providing flexibility in setting of resetting critical values of the ACT for clinical classification of patients into various strat. , such as 'Normal' 'doubtful' 'abnormal'.

In present study also all cases studied after 36 weeks of pregnancy showed accelerated clotting time less than 98 seconds.

Similar findings are also recorded by Manju Verma, Asha Pandey and Krishna Mukherji (1984) using other established parameters like period of gestation, birth-weight Crown-heel length and head circumference and was correlated with ACT.

In the present study also a birth weight of less than 1000 gms indicated prematurity and correspondingly the thromboplastic activity of amniotic

fluid was less as reflected by the higher ACT. As the birth weight increased period of gestation and maturity, the thromboplastic activity increased as reflected by a decrease in ACT.

Thus an inverse relationship of ACT with increasing fetal maturity is seen by all the parameters.

A similar relationship with L.S. ratio and ACT has been observed by Yaffe et al (1977).

The diagnosis of post-maturity remains a difficult problem. The thromboplastic activity of amniotic fluid (TAAF) increases with the gestational age. Based on this phenomenon, Yaffe, Esther Hay and Sodovsky (1981) reported that TAAF values of less than 42 seconds strongly suggested post-maturity. Pregnancy in which the TAAF value is between 42-45 seconds should be regarded as post-mature and high risk and TAAF of 45 seconds or more is not compatible with fetal post-maturity. In this present study also we found ACT below 46 seconds was associated with postmature foetus.

Thus enhancing the value of this test over other tests.

The present study has clearly demonstrated an increase in procoagulant activity in the amniotic fluid as was also shown by decrease in C.T. (Clotting time).

Similar observations were also showed previously in study of Weiner et al 1950, Rendelsten et al 1951, Phillips and Davidson 1972, Dube, Sharma et al 1975.

It is probable that a part of thrombokinase like activity comes from the decomposed material of epithelial cells of embryonic skin and amnion. A part of similar activity may also come from fetal urine in the amniotic fluid (Rendel stein et al 1951). An indirect evidence of this effect comes from our observation that the coagulant activity of amniotic fluid increased as pregnancy progressed.

The test described for the ACT probably measures the combined thromboplastic activity of the desquamated cells and phospholipids in the amniotic fluid.

All of several methods available to assess foetal maturity including LMP, Physical examination, Radiology, Ultrasound, Spectrophotometry, Amniotic fluid creatinine and cytology have in accuracies, deficiencies and require sophesticated equipment except shake test which is bedside.

ACT (Accelerated Clotting Time) is a very simple test. Its simplicity can be rivaled by shake test and perhaps by Nile blue staining method. However, shake test suffers from subjective difference in interpretation of stable bubbles and has a false positive and negative rates of 18 and 7% respectively (Shepard et al 1974). The nile blue staining method is simple but has wide transitional range and 15% false negative rate.

In present study ACT is very fast test yielding results immidiately. It doesnot require any sophesticated equipment.

This test can be done at bedside. The false positive rate is 2% and false negative 4%.

Hence it can be concluded that ACT can be used to detect foetal maturity more particularly post-datism.

Hence this information can be used for induction of labour and termination of prgenancy where a decision is to be taken that a point has been atained where infant survival and development may be more safe in a nursery than in utero.

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CONCLUSIONS

CONCLUSIONS

A study of Accelerated Clotting time in amniotic fluid was done in 70 patients. The results of these tests were analysed and their correlation to duration of pregnancy were studied. The gestational age of baby was determined by physical criteria.

From the present study following conclusions are drawn :-

1. Amniotic fluid has thromboplastic activity. In all of the sample studied addition of amniotic fluid to maternal blood causes decrease in the Clotting time. The control clotting time varied between 6 to 8 minutes. Whereas Accelerated Clotting was always below 3 minutes 20 seconds, showing significant increase in thromboplastic activity.

2. This thromboplastic activity increases with gestational age which can be detected by decrease in Accelerated clotting time. In all of the cases studied below 32 weeks of gestational age the accelerated clotting time ranged between 130-200 seconds i.e. always more than 2 minutes.

On the other hand in pregnancies ranging 32 - 36 weeks the accelerated clotting time was between 96 - 145 sec. Average being 117.67 sec. Clearly showing Linear fall in ACT with increasing gestational age.

3. Gestational age correlates well with fall of ACT. In the present study it was found that when gestational age varied between 28 - 32 weeks, the average ACT was 169.6 sec. and when gestational age was 32 - 36 weeks, average ACT was 117.67 sec.

In pregnancies between 36 - 40 weeks ACT was 80.44 sec. It clearly shows a negative correlation between gestational age and accelerated clotting time. By applying statistical methods the difference in between ACT of various groups was found to be highly significant ($P < .001$). Hence this rapid fall in ACT can very easily be used to determine foetal maturity.

4.(i) In our present study ACT of less than 98 seconds was compatible with foetus of 36 weeks or more

foetus of more than 36 weeks is taken as mature foetus with full functional lung maturity and survival of foetus Ex-utero.

Gestational age of 36 weeks or more as determined by ACT correlated well when gestational age of fetus was reconfirmed by physical criteria including sole creases, breast nodule diameter, scalp hair, ear lobe and genitalia.

False positive rate was only 2%.

(ii) Present study also concludes that an ACT of 46 seconds or less was associated with foetus more than 42 weeks. Post-mature foetus is associated with more complications and increased Unexplained perinatal mortality. Hence it is very important to detect post-maturity.

In present study there was no case when gestational age was more than 42 weeks and ACT of more than 46 seconds hence showing very valuable test to detect post-datism.

5. The present study shows that accelerated clotting time can serve as an index of foetal maturity which is a fast, simple, reliable and inexpensive method.

The equipment required is very simple and available in every pathology lab. which includes test-tubes, stopwatch and water bath.

The results obtained correlates well with other test like L.S. ratio and Nileblue sulfate test. These results also correlated well when gestational age was reconfirmed by physical criteria in the newborn.

It can be concluded that ACT which reflects the increasing thromboplastic activity can be utilized to estimate.

(i) Foetal maturity

(ii) Post - dated pregnancy

thus serve to decrease complications of premature induction of labour as well as postmature foetus.

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APPENDIX

APPENDIX

WORKING PROFORMA

Case No. MRD No.

Name Age/Sex

Address

Present History

Obstetrical History

Gravida

Parity

Abortion

Menstrual History

LMP (Last Menstrual period)

EDD (Expected Date of Delivery)

General Examination :

G.C.	P/A examination
Pulse	FH
B.P.	Presentation
Resp.	Lie
Temp.	FHS
Palor	P/V OS
Icterus	Cx
Cyanosis	Vx
Clubbing	Mem
Odema	Pelvis
Hydration	

METHOD OF OBTAINING AMNIOTIC FLUID

Accelerated Clotting time

Control Clotting time

Age

BABY :

Determination of gestational age by physical criteria

1. Sole creases
2. Breast nodule
3. Scalp hair
4. Ear lobe
5. Genitals

Baby weight

Head circumference

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